Fluorescence Polarization Immunoassays for Pesticides

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Abstract: Fluorescence polarization immunoassay methods for the detection of pesticides and their metabolites or degradation products are reviewed. Advantages and limitations for application to pesticide detection in environmental and food samples are discussed. The influence of the structure of fluorescent-labeled tracers and the affinity and specificity of antibodies on analytical performance is examined. The methods are simple, readily automated, and rapid (total time for assay of a water sample is about 1 min) with sensitivity of 1 - 10 ng/ml pesticide in 0.01 - 0.1 ml sample.

Key words: Fluorescence polarization immunoassay, pesticide, review.

INTRODUCTION

Pesticides are one of the major organic pollutants found in environmental and food samples. The wide and growing application of pesticides creates a need for simple and quick methods for their detection. Pesticide contamination may vary from vanishingly small levels up to very dirty samples with hundreds of mg per ml. The number of different pesticides and their metabolites or degradation products present in real environmental samples may range from one to several dozen and their distribution in different types of environmental or food materials may also be very variable. These factors mean that high numbers of sample measurements are required for correct evaluation and statistical interpretation of pesticide contamination results. The analytical methods applied must ideally be highly versatile, with high throughput, a wide operating range, and universal applicability.

The techniques currently applied for pesticide determination are primarily chromatographic methods, involving various detection principles. A number of reviews covering pesticides are published every year and recent and excellent examples include those by Nistor and Emneus [1] and Clement *et al.* [2]. The chromatographic methods have sufficient sensitivity for pesticide detection, but they are time consuming and involve expensive instrumentation. The need for sample extraction or extensive clean-up prior to analysis [3, 4] is another limitation in routine applications, though the collection and processing of solid samples relevant to environmental and food testing may often represent the most time-consuming and costly part of any analytical method. New trends in chromatographic methods include the application of antibody, either for affinity sample purification prior to analysis [5], or for immunodetection [6].

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In recent years, immunoassay methods have been increasingly applied to simpler and quicker pesticide detection. Excellent reviews can be found in any year in the last decade. Amongst the most recent, which include references to earlier reviews, are those by Dankwardt [7] and Niessner and Knopp [8]. Most of these methods are enzyme linked immunosorbent assays (ELISA) [9] with photometric detection, but the end point may also be chemiluminescent, fluorescent or electrochemical [10]. Relative to the chromatographic techniques, immunoassays have advantages that are particularly relevant for pesticide screening and analysis: these include the performance of direct analysis of large numbers of samples, and a common methodology which is applicable to a wide range of pesticide types. Many immunoassays will detect the presence of any one of a range of structurally-related analytes, hence they may find application as class-specific screens, while not providing the capability to identify and quantitate the specific pesticide which has caused the response.

The recognition of the practical advantages of ELISAs has led to their commercial availability and increasing use [1, 11]. The equipment required for ELISA is relatively inexpensive as compared to that for most chromatographic methods, but ELISA is a multi-step procedure, which requires separation of free and bound immunoreagents and involves several washing steps (though these are readily automated) as well as an enzymatic end-point detection step [12].

A very promising direction for assay simplification is the development of immunosensors, where the recognition element is usually antibody [13], although natural receptors may also be also used in pesticide sensors [14]. Other types of recognition elements are aptamers, which are artificial nucleic acid ligands that can be generated against amino acids, drugs, proteins and other molecules including pesticides [15]. Different kinds of immunosensors in clinical chemistry have been reviewed [16] and those for pesticides detection have been described in a recent special issue of *Anal. Bioanal. Chem.* [17]. At their current stage of development, they may suffer from low precision and restricted dynamic range. The main limitation on the routine use of sensors is the requirement for regeneration, or even

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entire replacement, of the recognition surface following each analysis.

Other recent developments in pesticide detection by immunoassay include the performance of assays in flow systems [1] and their miniaturization [18]. The miniaturized assays are usually the so-called "mix-and-measure" type of homogeneous (non-separation) assays, such as scintillation proximity assays, fluorescence correlation spectroscopy based assays and fluorescence resonant energy transfer assays. Research into the application of so-called micro-chip technology to pesticides has grown in the last 2 - 3 years [19], but this technique is still in the development stages and needs additional expensive equipment.

The most generally promising means for simplification of immunoassays for routine applications is a shift from heterogeneous methods (with separation) to homogenous assays (without separation). Such approaches could improve the practicability of so-called HTS (high throughput screening). HTS for drugs were reviewed recently [20] and it may be envisaged that the same principles could be applied for pesticides in the near future. New homogeneous immunoassays for pesticides developed in the group of Gauglitz are based on the "fluorescent immunoassays by energy transfer" technique [21]. This type of assay can be significantly miniaturized in a flow system or in nanotiter plates [22]. Recent developments in this area are covered in the review by Agbaria et al. [23].

Amongst the immunoassay methods, fluorescence polarization immunoassay (FPIA) fulfills almost completely the requirements for simple, reliable, fast and cost-effective analysis. FPIA is a homogeneous assay based on the increase in the polarization of the fluorescence of a small fluorescent-labeled hapten (tracer) when bound by a specific antibody. The label used is typically fluorescein. If the sample contains unlabeled pesticides, they will compete with tracer for binding with antibody and the polarization signal will decrease. The theory and application of FPIA has been described in several books on immunochemical techniques [24-26]. One of the most comprehensive and pioneering reviews on FPIA and its applications, which includes 195 references, was published in 1989 [27]. Since then, several reviews for FPIA of pesticides and biologically active compounds [28, 29] and other compounds including toxins [30] have appeared. For this reason, this review will focus on the advances in FPIA and its application for the detection of pesticides and related compounds in the last few years.

To summarize this brief introduction, the primary requirement for wider success of screening detection of pesticides is the simplification of the assay, which should reduce both the cost and time of testing. From the authors' point of view, homogeneous immunoassays, which are very simple and fast because they do not need any separation of immunoreagents or washing steps, are amongst the most promising ones in this respect.

ADVANTAGES AND LIMITATIONS OF FPIA

FPIA has many of the advantages common to all immunoassays, including:

- Universal applicability, since antibodies may be raised against almost all organic molecules, including all pesticides. Recently FPIA have been developed for small and weakly immunogenic compounds including the organophosphate pesticide parathion-methyl [31], and environmental contaminants which comprise little more than linear hydrocarbon chains such as the detergents nonylphenol [32, 33] and LAS (linear alkylbenzenesulfonate) [32, 34], and BTEX (benzene, toluene, ethylbenzene, xylenes) [35].
- Versatility in development either of specific assays for a particular analyte molecule or "class-specific" screening tests. Thus, by appropriate chemistry in the design of immunogens, either unique or common structural features may be exposed to the host animal's immune system so as to elicit antibodies with the desired specificity, for example for FPIA of DDT and its metabolites [36, 37]. Assay specificity may also be modulated by alteration in the structure of the tracer reagent as described below for FPIA of the 2,4-D family of chlorinated phenoxyacid herbicides [38, 39] and s-triazines [40, 41]. Assays may be designed for quantitation of a single specific pesticide, or for detecting a group of structurally related pesticides and all of their environmental degradation products or metabolites.
- A common methodology that can be applied to all analytes, which facilitates the development and automation of instrumental methods.

Moreover, FPIA have several unique features specifically related to the use of a fluorophore (usually fluorescein) as the label and to the measurement of fluorescence polarization as the analytical signal:

- A homogeneous assay protocol with no separation step. Assay time is limited by pipetting and the antigen-antibody reaction rate, which is fast. Measured rate constants for the binding of small haptens by antibody are generally in the region 10^7 to 10^8 L mol^{-1} s^{-1}, which implies that the only limitation is the rate of diffusion of the reactants together in solution [42]. This facilitates quick screening tests and automation.
- The polarization of fluorescence (P) is an inherently ratiometric parameter, being defined in terms of the intensities of the vertically (V) and horizontally (H) polarized components of the fluorescence emission according to the equation $P = (V - H)/(V + H)$. This cancels out the effect of variations in instrumental sensitivity, e.g. excitation light intensity fluctuations, and leads to greater stability and reproducibility of the end-point measurement over time.
- Fluorescence is a versatile detection method. It may be implemented with simple instrumentation like the FPM (Diachemix Corp., USA) and BEACON 2000 (PanVera Corp., USA) instruments or adapted to microtiter plates or other reading formats as in the Tecan Polarion (Tecan UK Ltd., UK), Victor (LKB-

Pharmacia, Sweden), or PolarStar (BMG LabTechnologies GmbH, Germany).

- Fluorescein has excellent chemical stability, such that FPIA reagents are stable for years.
- FPIA is readily automated and gives very reproducible and stable assays (provided that measurement temperature is controlled, since fluorescence polarization will decrease with increase of Brownian molecular rotation rates). The classic example is the Abbott TDx Analyzer, which was developed and introduced in the 1970s to provide a fully automated system for therapeutic drug monitoring and drug of abuse screening. The TDx met with outstanding commercial success owing to its reliability and the wide range of assays available. Numerous FPIAs have since been adapted to this instrument, by Eremin's group in Moscow and by others, and many of these are referred to in Table 1.

As with any analytical method, FPIA has some disadvantages, or more correctly limitations:

- The sensitivity of FPIA is not as good as for ELISA. The minimum detectable quantity in an FPIA is about 0.1 - 10 ng analyte and the working range for FPIA is generally in the ng/ml to µg/ml range (Table 1). In the European Community, the maximum levels for a single pesticide and for the total of all pesticides in drinking water are 0.1 ng/ml and 0.5 ng/ml, respectively. Pesticides may be found in environmental water samples up to several ng/ml [12, 13]. Residue levels in waste waters could be at the

Table 1. Minimal Detectable Quantity and Working Ranges for FPIA of Pesticides and Related Compounds

Compound	Minimal detectable quantity (ng)	Range of detection (µg/ml)	Reference
Atrazine	0.1	0.001-1	28, 43, 44
Simazine	0.15	0.001-1	28, 40
Triazine	0.25	0.001-1	41, 45
2,4-Dichlorophenoxyacetic acid (2,4-D)	5	0.1-100	28, 38, 46-49
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	4	0.2-10	28, 50-52
2,4-D 2,4,5-T	0.01 0.5	0.0001-1 0.01-0.2	53
2,4-D 2,4,5-T	0.08 0.5	0.001-0.1 0.01-1	54
[(±)-2-(2,4-Dichlorophenoxy)propanoic acid (dichlorprop)	30	0.01-100	55
2-Methyl-4-chlorophenoxyacetic acid (MCPA)	500	2-200	39
2-Methyl-4-chlorophenoxybutyric acid (MCPB)	50	1-100	56
Triclopyr	2.1	0.86-100	57
Pentachlorophenol	0.5	0.01-10	58
DDT specific DDT class	12 3	0.02-1 0.006-1	37
Isoproturon	4	0.5-10	59
Methabenzthiazurone	0.4	0.02-5	60, 61
Metsulfuron-methyl	0.25	0.03-1	62
Chlorsulfuron	0.5	0.01-1	63
Acetochlor	0.45	0.05-5.5	64
Propanil	0.025	0.001-0.1	65, 66
2-Aminobenzimidazole (degradation product of benomyl)	0.3	0.001-0.1	67
Paraquat	1.5	0.025-2	68
Parathion-methyl	1.5	0.025-10	31
BTEX (benzene, toluene, ethylbenzene, xylenes)	13	10-1000	35
Nonylphenol	400	20-160	32, 33
LAS (linear alkylbenzenesulphonate)	250	3-85	32, 34

solubility limit of the pesticides involved. For these reasons, the sensitivity of FPIA may suffice for direct detection of pesticides in water samples. Examples of the application of FPIA to extracts from food and soil samples are found in some of the references listed in Table 1.

- FPIA is sensitive to interference from endogenous fluorophores in samples or from light scattering. These effects may be corrected by measuring the vertical and horizontal signal components of the background fluorescence or scatter and subtracting from the corresponding signals from the immunoassay mixtures before calculating fluorescence polarization. This procedure is automated in the Abbott TDx Analyzer and is a major factor underlying its accuracy and dependability.
- Non-specific binding of the FPIA tracer by components of some sample matrixes may lead to spurious increase in polarization signal. In other words, the FPIA method may be matrix dependent. These matrix effects may in some cases be eliminated by appropriate formulation of the PFIA assay diluent medium to prevent the non-specific binding effects.
- The FPIA needs special instrumentation for fluorescence polarization measurement and is applicable primarily for detection of small molecules because of the requirement for a significant increase of the polarization signal upon antibody binding of the labeled reagent.
- The tracer is a vital reagent required for the implementation of any FPIA method. Various approaches to the design and chemical synthesis of fluorescein-labeled analogs of pesticide analytes are described in the references listed in Table 1. In general, the labeling of analyte molecules will affect their binding by antibody, but in appropriate circumstances this can be exploited to optimize FPIA systems, notably in terms of their sensitivity as discussed in more detail below.

It is essential to have a clear understanding of the limitations of any currently used analytical technique, because today's instrumental developments are so fast that the disadvantages of some techniques might be obviated in the very near future. A recently developed assay of 2,4-D using a new tracer and optimized antibody and instrumentation shows that sensitivity may be extended down to 0.1 ng/ml [53].Two main developments in FPIA are the single-reagent and stopped-flow techniques. Additional improvement of FPIA performance could be made by use of the sample in a water-miscible organic solvent or assay in reverse micellar systems of surfactants in non-polar organic solvents.

Performance Characteristics of FPIA

FPIA standard curves are constructed by plotting the concentration of analyte against the fluorescence polarization, often in millipolarization (mP) units, or against the ratio of polarization to that of the maximum signal from the zero-concentration standard (mP/mP_{max}, equivalent to B/B_0 in ELISA). Typical standard curves, for FPIA of 2,4-D and 2,4,5-T [53], are presented in Fig. 1 (see Table 1 for pesticide abbreviations). The results were obtained after choosing the best available antibodies and optimization of tracer structures. The 2,4-D system employed a monoclonal antibody against 2,4-D and fluorescein-labeled MCPA as tracer; the 2,4,5-T system employed a rabbit polyclonal antiserum and fluorescein-labeled 2,4,5-T as tracer [53]. The sensitivity for 2,4-D is about 0.1 ng/mL which is 1-2 orders better than for 2,4,5-T. The difference in sensitivity could be explained by the optimal combination of high affinity antibody and tracer in the 2,4-D system. From Table 1, it is apparent that this particular 2,4-D assay is the most sensitive reported FPIA.

The sensitivity of an FPIA system depends on both the relative and absolute magnitudes of the binding affinity constants of the tracer and of the pesticide analyte for the antibody reagent. As discussed in detail by Krikunova et al.

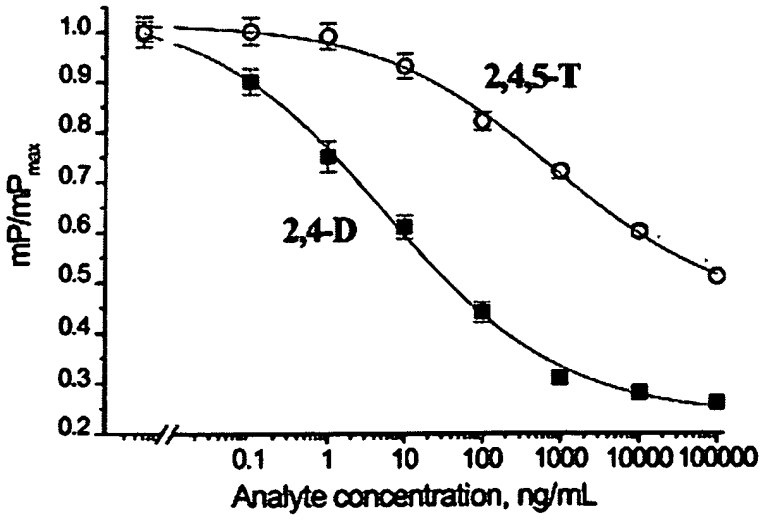

Fig. (1). FPIA standard curves for 2,4-D and 2,4,5-T [53].

[53] and Hatzidakis et al. [54], the lowest detection limit in a competitive immunoassay is generally obtained by employing high-affinity antibody and by achieving the most effective competition between the analyte and the tracer. This generally implies that the tracer should have affinity for the antibody close to that of the analyte. Homologous tracers (in which the labeled analyte moiety is the same as that used to prepare the immunogen used to elicit the antibody reagent) generally bind their corresponding antibody better than the free analyte. This occurs because immunogen preparation involves a chemical "bridge" structure to link the analyte with the immunogenic carrier protein. The resulting antibodies have affinity towards the bridge structure as well as towards the analyte itself. The tracer structure will generally incorporate a bridge between the analyte and the fluorescein label and this contributes to extra binding affinity for the tracer as compared with the free analyte. Heterologous tracers (with a different labeled analyte moiety) will have lower binding affinity which may be closer to that of the analyte. Thus it may be beneficial if the structure of the analyte residue in the labeled antigen is modified so as to decrease the affinity constant, thereby attaining higher assay sensitivity. In the 2,4-D example system [53], the most sensitive assay for 2,4-D was achieved using a 2,4-D antibody and a tracer prepared with MCPA in place of 2,4-D.

From the duplicates shown in Fig. 1 it is seen that the precision of FPIA is high for both analytes, as is the case for most FPIA methods, with coefficients of variation (CV) in the region of 5% or lower being achievable. The assay CV may generally be determined from the results of up to 10 replicates; it is a composite of various factors including the precision of reagent pipetting.

Single-Reagent FPIA

FPIA is very simple in performance but, nevertheless, may be further simplified by using the single-reagent (SR) format, which was originally developed for FPIA of abused drugs [42]. The SR is a pre-equilibrated solution of antibody with tracer, which can be used as a direct immunoreagent, with measurement of displacement of tracer from immunocomplex after sample addition. The antibody and tracer pair for a SR format must be chosen such that it has fast dissociation kinetics. The change of polarization signal depends on the concentration of an analyte in the sample and the time of displacement. This SR-FPIA provides an exceptionally simple, one-step immunoassay method. Sample is added to the single reagent and displacement of tracer from the immunocomplex is measured after brief incubation. For semi-quantitative assays (in which a "positive" or "negative" result is provided relative to a pre-determined threshold level of analyte), results may typically be obtained after 2 to 3 minutes of incubation, while a precise SR FPIA standard curve may generally be obtained after 15 to 30 min. In some cases, SR has proved to be more stable than corresponding individual solutions of tracer and antiserum, presumably because of antibody protection of the bound tracer. Moreover, the standard curve is stable at least one week and may be used for quantitative detection without recalibration at each assay performance [35, 44, 61].

The performance of SR-FPIA is very simple and could be classified as a one-step method. For example, for FPIA of atrazine [44], 100 µl of standard or sample was mixed with 1 ml of SR and fluorescence polarization was measured on the Abbott TDx Analyzer in Photo Check mode without any pre-incubation. Total time for measurement of up to 10 samples on this instrument is 7 min. The SR was prepared by pre-incubation of tracer and antibody solutions in appropriate concentration. The SR-FPIA reagents and FPIA standard curves are very stable. When SR was prepared and stored at room temperature, the FPIA calibrators run daily over one week gave the same analytical responses (Fig. 2) [44]. It is clear that the SR could be even more stable at 4°C and could be used for several months for quantitative determination of analyte concentrations without preparation

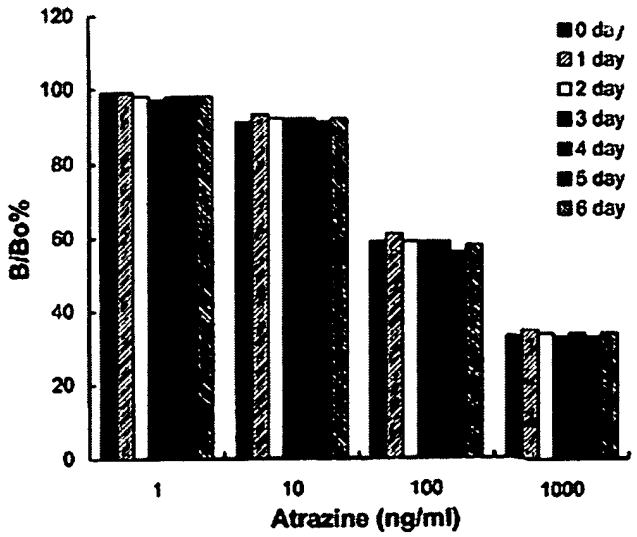

Fig. (2). Weekly stability of SR-FPIA for atrazine determination using SR stored at room temperature [44].

of standard curves, which is a requirement for most immunoassay methods.

SR FPIAs have shown their usefulness in drug abuse screening and in therapeutic drug monitoring [42,69,70], and they have recently been developed for detection of toluene [35], methabenzthiazurone [61], atrazine [44], and some other pesticides (unpublished results).

Stopped-Flow FPIA

As in any homogeneous immunoassay the main limitation of FPIA, when applied to the analysis of real samples, is its relatively low sensitivity. This is a result of the relatively high level of the background signal, which is caused partly by scattered light and partly by fluorescent components of the sample matrix. An approach to avoid or minimize this effect is to use the initial rate of the immunochemical reaction as an analytical parameter instead of the signal obtained when the reaction reaches or is close to equilibrium. The initial rate is determined from the time course of the change in the analytical signal as the reaction proceeds. This change is entirely due to change in the signal from the tracer reagent as it participates in specific antigen-antibody binding reactions. The background signal is constant and therefore makes no contribution to the initial rate.

Since the competitive antigen-antibody binding reactions are usually very fast, kinetic data are best obtained using the stopped-flow (SF) mixing technique. The SF method allows measurements shortly after mixing the reagents and, in addition, enables the automation of this step of the analytical process. The details of stopped-flow FPIA are described in the corresponding article in this issue.

The first applications of SF FPIA in environmental and food analysis were made recently by development of methods for the determination of 2,4-D [71] and atrazine [72] in river water, orange juice and white wine samples. The SF FPIA method reduces the time of reactant manipulations and shows a high sample throughput, as the measurement step takes only one second. Moreover, compared with the conventional FPIA method, the kinetic methodology allows direct analysis and decreases the detection limit 10-fold because the dynamic measurement is obtained at the beginning of the reaction between the tracer and antibody, thus avoiding or minimizing the effect of background signal and the potential interferences from the sample matrix.

FPIA in Organic Solvent

Reverse micellar systems of surfactants in non-polar organic solvents are homogeneous organic media which are able to solubilize biologically active substances such as antibodies, giving optically clear solutions. Recently an octane-compatible FPIA method was developed to analyze pesticides using specific antibodies solubilized in reverse micelles of sodium bis(2-ethylhexyl)sulfosuccinate surfactant in octane (Aerosol OT). The advantage of reverse micellar systems is that the analyte can be added when dissolved in a non-polar organic solvent. The feasibility of FPIA for 2,4-D [73] and propazine [74] in reverse micellar systems has been described. The detection limit of FPIA in reverse micellar systems is comparable with that in aqueous medium.

Another alternative for improvement of FPIA performance is use of the sample in a water-miscible organic solvent such as methanol, acetonitrile or others. In FPIAs of the pesticides 2,4,5-T [47], acetochlor [64], parathion-methyl [31] it was found that up to 10% of organic solvent in the reaction mixture could be tolerated by the specific antigen-antibody reaction. This means that the analyte could be directly measured in solvent samples, and the extraction of antigen from complex food or environmental samples could therefore be simplified.

INFLUENCE OF STRUCTURE OF IMMUNOREAGENTS IN FPIA

The antibody is a key determinant of the specificity and sensitivity of immunoassay methods. For example, the use of different monoclonal antibodies for DDT permitted the development of a specific FPIA or a broad-spectrum assay for DDT and its metabolites [36]. FPIAs for nonylphenol were developed either with a specific antibody, exhibiting only minor cross-reactivity towards mono- and bis-substituted phenolic compounds, or with an antibody recognizing p-substituted phenols to obtain a broadly specific FPIA [33]. The sensitivity of FPIA for MCPA was maximized by using tracer synthesized from MCPA and a polyclonal antibody developed against 2,4-D [39]. The affinity of antibodies to atrazine, which can be determined by fluorescence polarization methods [75], was found to be strongly dependent on the immunogen used for antibody production.

However, the structure of the tracer also affects these assay characteristics. Using the same antibody, the analytical parameters of FPIA may be optimized by careful choice of the tracer's structure [28]. For example, different triazine tracers were used for optimization of FPIA of atrazine [43, 44]. In several systems, sensitivity has been found to be greatest using the shortest chemical bridge between the antigen and the fluorescent label [29, 31, 66]. Labeled antigens that were structurally homologous or heterologous to the primary target analytes were investigated and some FPIA systems were found to be more sensitive when structurally heterologous tracers were used [28]. These principles are illustrated in FPIA developed recently for 2,4-D and 2,4,5-T [51, 52].

The bridge itself may also be modulated. Assays for 2,4-D [54] were developed using a monoclonal antibody raised against 2,4-D attached to the ε-amino groups of lysine residues of the immunogenic carrier protein. Two tracers were prepared using lysine as the bridge structure: one with 2,4-D attached to the ε-amino and fluorescein attached to the α-amino group, the other with 2,4-D attached to α-amino and fluorescein attached to the ε-amino group. With the latter tracer, 7.6 times better sensitivity was obtained and this was attributed to the reversed orientation of the bridge, leading to limited recognition by the antibody.

In many cases, pesticide FPIAs have been developed utilizing antibodies that had already been produced for

application in ELISA. The fluorescein-labeled tracers were then specially synthesized for FPIA use. But sometimes, the antibodies were originally produced for FPIA development (acetochlor [64], chlorsulfuron [63], propanil [66], nonylphenol [33], and others) and later applied for ELISA or other immunochemical techniques.

Some attempts to develop FPIAs using antibodies from ELISA have failed, for example for p-nitrophenol, dioxins, and some organophosphates. However, the antibodies involved in these cases could not be applied in the direct ELISA technique, only for indirect ELISA. Different immunoassay formats may depend on different antibody kinetic characteristics for optimal performance. The FPIA is a competitive immunoassay method and this may have particular significance for antibodies against very small or highly hydrophobic pesticides. As a result, the development of suitable antibodies and the design and choice of fluorescein-labeled tracers for the FPIA of pesticides remain objectives for research.

FPIA for Pesticides

Colbert and Coxon were the first who described FPIA for detection of a pesticide, in 1988 [68]. They developed a method for paraquat in serum samples and adapted this assay for use in the TDx Analyzer. It should be noted that Dandliker and co-authors, in their pioneering work on development of the principle of FPIA, described a method for 2-aminobenzimidazole, which is the main degradation product of the pesticide benomyl [67]. Potentially, the detection of 2-aminobenzimidazole could be used for preliminary screening for benomyl. Similarly, FPIA of the pesticide 2,4,5-T, which is one of the precursors of polychlorinated dibenzo-p-dioxins, has been proposed as a screening method to provide preliminary evidence of dioxin contamination [52, 53, 58].

In Table 1, successful applications of FPIA for different pesticides and some environmental contaminants developed during the last ten years in the laboratory of Eremin's group at Moscow State University (Russia) and Sanchez's group in Spain are given together with brief details of the assay performance characteristics.

FPIA for Related Compounds in Environmental Monitoring and Food Control

FPIA techniques may be useful not only for pesticide control, but also as described in detail elsewhere in this issue, for environmental screening for metals such as cadmium [76] and lead [77], endocrine disruptors like estrogens [78, 79], toxins like aflatoxins [80], fumonisins [81], deoxynivalenol [82] and veterinary drugs such as sulphamethazine [83], digitoxin [84], and aminoglycoside antibiotics [85]. Applications to nonylphenol, LAS, and BTEX have been mentioned above.

Related Fluorescence Techniques for Pesticide Detection

In some special cases, the interaction of fluorescein-labeled antigen and specific antibody may result in a change (increase or decrease) in the intensity of fluorescence. This phenomenon, which was well known for drugs [86], was extended to pesticide assay by Matveeva et al. in developing quenching fluoroimmunoassays of 2,4-D [73], propazine [87], atrazine [88], and pyrethroid metabolite [89]. A homogeneous phosphorescent immunoassay for the detection of polychlorinated dibenzo-p-dioxins has also been described [90].

The same reagents used for FPIA of pesticides may be applied in flow immunoassay systems. Fluorescence based methods utilizing restricted access columns were developed for atrazine by Onnerfjord *et al.* [91,92] and for p-nitrophenol by Emneus and co-authors [93]. Such systems have potential for non-stop monitoring of water in environmental testing. The trace detection of explosives like 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) using a membrane-based displacement fluoroimmunoassay in a flow system was described by the group of Kusterbeck [94-97].

The analytical signal from the fluorescein label may also be registered in different ways. A homogeneous phase-modulation fluoroimmunoassay for 2,4-D [98] was described by Sanchez and this group also developed an ELISA for 2,4,5-T with fluorescence detection of peroxidase [99]. Selective determination of carbaryl and benomyl was possible by fluorescence polarization [100] but this method is different from FPIA, because it does not employ antibodies but is based on the fluorescence of the pesticides themselves. Other pesticides have intrinsic fluorescence properties by which they may be directly detected [101]. The selective binding of polychlorinated biphenyl (PCB) congeners by a monoclonal antibody was used for PCB detection by kinetic exclusion fluorescence immunoassay [102].

More selective detection methods for pesticides may be based on photochemically induced fluorescence. For example, photochemical-fluorimetric methods were described for chlorophenoxyacid herbicides in batch mode [103,104] and in a flow system [105,106]. Sulphamethoxazole was detected by photochemically induced fluorescence in drugs and milk [107]. Chemiluminescence determination of carbofuran and promecarb by flow injection analysis using two photochemical reactions has been described [108]. Pesticide detection by fluorescence and luminescence techniques has been reviewed by Coly and Aaron [109,110].

In recent years, wide application has been proposed for molecularly imprinted polymers (MIPs) as recognition elements. MIPs are increasingly used for on-line pre-concentration by solid-phase extraction and on-line cleanup of samples for pesticide detection [111-114]. Moreover, MIPs could be used as so called "synthetic antibodies" and have been substituted as such in the "enzyme-linked molecularly imprinted sorbent assay" by the group of Danielsson [115-117]. Recent applications of MIPs in enzyme-linked and fluorescent assays have been reviewed [118,119].

CONCLUSIONS

FPIA for pesticides is a simple, fast, accurate and cost-effective method for environmental and food safety control.

Moreover, this technique is versatile in its applications and easy to automate. Its most significant disadvantage relative to ELISA is limited sensitivity. As compared with chromatographic methods, screening-type FPIAs are unable to differentiate the identities of the analytes involved when a positive response is found, and they may not produce an equal response to each individual analyte. However, with further technical developments, FPIA could become dominant in pesticide detection in the near future. Such developments may include the general tendency in analytical methods towards miniaturization. FPIA has been successfully miniaturized, as described in recent reviews [120-121], and applications to pesticide detection may be anticipated. Expert systems provide another gateway to the future in food analysis [122].

ABBREVIATIONS

FPIA	=	Fluorescence polarization immunoassay
ELISA	=	Enzyme linked immunosorbent assay
HTS	=	High throughput screening
SR	=	Single-reagent
MIP	=	Molecularly imprinted polymers
DDT	=	1,1,1- trichloro-2,2-bis(p-chlorphenyl)ethane
2,4-D	=	2,4-dichlorophenoxyacetic acid
2,4,5-T	=	2,4,5-trichlorophenoxyacetic acid
MCPA	=	2-methyl-4-chlorophenoxyacetic acid
MCPB	=	2-methyl-4-chlorophenoxybutyric acid
PCB	=	Polychlorinated biphenyl
BTEX	=	Benzene, toluene, ethylbenzene, xylenes
LAS	=	Linear alkylbenzenesulphonate
TNT	=	2,4,6-trinitrotoluene
RDX	=	Hexahydro-1,3,5-trinitro-1,3,5-triazine

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